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CONTRACT TITLE: Recognition of DNA by EcoRI Restriction Endonuclease and Methylase

CONTRACT DATES: 1 February 1988 - 1 February 1991

RESEARCH OBJECTIVE: To elucidate the factors underlying sequence specific recognition of DNA by proteins (EcoRI Restriction Endonuclease and Methylase), drugs (echinomycin), and the third strand in DNA triplexes.

ACCOMPLISHMENTS and SIGNIFICANCE :

NMR Studies of Echinomycin-DNA Complexes

We have studied the interaction of echinomycin, a cyclic octadepsipeptide antibiotic, with DNA oligonucleotides. These complexes were studied by two-dimensional proton NMR techniques. Echinomycin binds in a sequence specific manner to DNA by bis-intercalation, with a preferred 4 base binding site centered on CpG. Crystal structures of echinomycin and the related Triostin A complexed to DNA hexamers and octamers (solved in the Rich laboratory) showed that base pairs adjacent to the echinomycin binding site were Hoogsteen rather than Watson-Crick base paired. However, the evidence for Hoogsteen base pairing in complexes of echinomycin with DNA restriction fragments has been equivocal (Waring and Dervan laboratories). We have been studying the solution structures of echinomycin-DNA complexes in order to determine (1) if Hoogsteen base pairs are forming adjacent to echinomycin binding sites in solution and (2) the sequence specificity of echinomycin binding in terms of the structures formed.

In our initial studies, we looked at complexes formed between echinomycin and the DNA oligonucleotides $[d(CGTACG)]_2$ and $[d(ACGTACGT)]_2$ (*Proc. Natl. Acad. Sci. USA* **86**, 3006 (1989)). Two echinomycins were found to bind per duplex, as in the crystal structure with d(CGTACG), with the quinoxaline rings of the echinomycin bracketing the CpG steps. The surprising result we obtained was that the conformation of the complexes changed with temperature. At low temperatures, for the octamer duplex, both the terminal and the interior AT base pairs were Hoogsteen. However, as the temperature was raised, the interior AT Hoogsteen base pairs were destabilized and began to exchange between a Hoogsteen and an open (or possibly Watson-Crick base paired) state. The Hoogsteen base pairs at the ends of the duplex remained stable up to at least 45 °C.

This work was followed by a detailed comparison of the $[d(ACGTACGT)]_2$-2echinomycin and the $[d(TCGATCGA)]_2$-2echinomycin complexes (*Biochemistry*, **30**, 2483 (1991)). We assigned almost all of the drug and DNA resonances and have detailed contacts between the drug

and the DNA in both complexes. For the latter complex, the fully saturated drug-DNA complex shows two drugs bound per duplex at the CpG binding sites, but no Hoogsteen base pairs are formed under any conditions and the interior A·T base pairs are stabilized by drug binding.

From these studies, we have concluded that no single structural change in the DNA can explain all of the chemical and nuclease footprinting results which have been obtained on echinomycin bound to restriction fragments of plasmid DNA. We also conclude that even for sequences where Hoogsteen base pairs are observed in the crystal structure and at low temperatures in solution, Hoogsteen base pairing is probably not the relevant conformation *in vivo*.

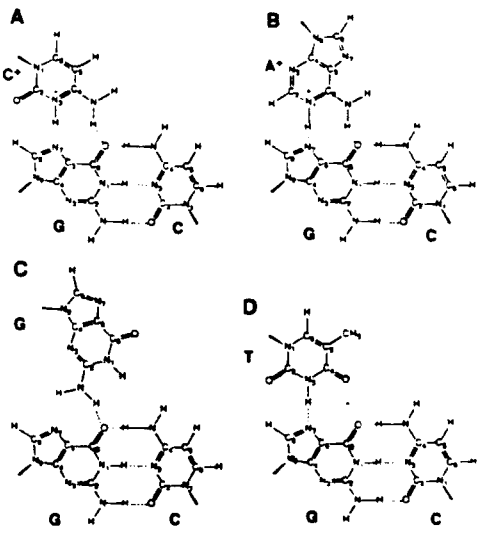
NMR Studies of Triplex Formation

We have studied the formation of DNA triplexes from DNA oligonucleotides. In general, these are formed from one purine and two pyrimidine strands, but there are some reports of DNA triplexes formed from other sequences. DNA triplexes are currently of great interest because of (1) evidence that such structures may be forming as intramolecular triplexes *in vivo*, and may play a role in genetic regulation and (2) potential therapeutic applications of intermolecular triplexes to repress transcription.

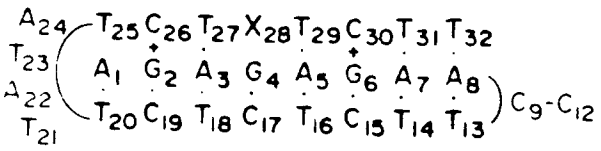
In our initial studies, we investigated the complexes formed by $d(GA)_4$ and $d(TC)_4$. This work was reported in *Nature* (**339**, 637 (1989)) and *Biochemistry* (**28**, 7859 (1989)). We were able to show unambiguously that the second pyrimidine strand bound in the major groove via Hoogsteen base pairs. This base pairing scheme requires protonation at the N7 of cytosine, and these protonated iminos were observed.

In our more recent work, we have investigated the structures of triplexes formed from folding of a single strand to form an intramolecular triplex. The first oligonucleotide we looked at was a 28 base oligonucleotide d(GAGAGAA<u>CCCC</u>TTCTCTC<u>TTT</u>CTCTCTT) where the underlined bases form the loops (*Nature* **345**, 836 (1990)). We found that this molecule did indeed fold to form an intramolecular triplex. This work was followed by a more detailed study of related 31 and 32 base oligonucleotides. A 32 base oligonucleotide shown below, where X=A,G,C, or T, was used to study the sequence specificity and stability of triplex formation. We found that all X·G·C triplets formed and the triplexes containing them had the relative stabilities C·G·C>>A·G·C>G·G·C>T·G·C. The hydrogen bonding scheme for all of these was determined (*Science* **254**, 270 (1991)).

$$\begin{matrix} A_{24} \\ T_{23} \\ A_{22} \\ T_{21} \end{matrix}\Bigg(\begin{matrix} T_{25}\,C_{26}\,T_{27}\,X_{28}\,T_{29}\,C_{30}\,T_{31}\,T_{32} \\ A_1\cdot G_2\cdot A_3\cdot G_4\cdot A_5\cdot G_6\cdot A_7\cdot A_8 \\ T_{20}\,C_{19}\,T_{18}\,C_{17}\,T_{16}\,C_{15}\,T_{14}\,T_{13} \end{matrix} \Bigg) C_9\text{-}C_{12}$$



Jeffrey Pelligon, UCLA, 1991



Accomplishments

- Obtained NMR evidence for intramolecular triplex formation
- Studies X.G.C triplets (X=C,G,A,T) and determined stability, base pairing scheme

Objective

- Study structure and sequence specificity of intramolecular DNA triplexes

Significance

- Single-strand folds to form intramolecular triplex
  - — Model system for H-DNA
  - — Minimum loop size 4 bases
  - — Some triplex forms at neutral pH
- Useful for studying sequence specificity of triplex formation

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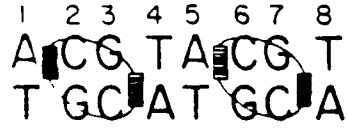

Accomplishments

- Determined binding mode & base pairing scheme for complexes shown above
- Determined that structure formed depends on sequence

Objectives

Echinomycin Binding to DNA

- Determine if Hoogsteen bp form in solution
- Study sequence specificity of complex formation

Significance

- Structure and stability depend on sequence
- Hoogsteen bp probably not relevant *in vivo*